

# Electrochemical sensing of NADH on NiO nanoparticles-modified carbon paste electrode and fabrication of ethanol dehydrogenase-based biosensor

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**Abstract** In this work, an electrochemical  $\beta$ -nicotinamide adenine dinucleotide (NADH) sensor based on a carbon paste electrode modified with nickel oxide nanoparticles (NiONPs) was developed. The key highlights of this work are ease of preparation of the NiONPs-modified carbon paste electrode (NiONPs/MCPE), and its high sensitivity to NADH. The electrochemical characterization of NiONPs/MCPEs was performed via cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The electrochemical oxidation response of NADH was investigated by differential pulse voltammetry and chronoamperometry. The results indicated that the electrocatalytic effects of NiONPs on the response current of NADH significantly facilitated the electron transfer and improved the performance of the biosensor. Compared to bare carbon paste electrode (BCPE), the oxidation potential was shifted toward more negative potentials and the oxidation current was increased remarkably. Under optimum conditions, NADH could be detected in the range from  $1.0 \times 10^{-4}$  to  $1.0 \text{ mmol L}^{-1}$  with lower detection limit ( $0.05 \text{ } \mu\text{mol L}^{-1}$ ). The proposed NADH sensor demonstrated fast and reproducible response. Furthermore, an ethanol biosensor was prepared using NiONPs and  $\text{NAD}^{+}$ -dependent alcohol dehydrogenase enzyme giving linear responses over the concentration range of 1.6 and  $38 \text{ mmol L}^{-1}$  of ethanol.

**Keywords** NADH sensing · NiO nanoparticles · Carbon paste electrode · Electrocatalytic oxidation · Ethanol

## 1 Introduction

The electrochemical sensing of  $\beta$ -nicotinamide adenine dinucleotide (NADH) has received great interest, owing to its importance as a cofactor in many enzymatic reactions of  $\text{NAD}^{+}$ /NADH-dependent dehydrogenase-based biosensors [1]. Nevertheless, direct oxidation of the  $\text{NAD}^{+}$ /NADH couple is not high ( $-0.56 \text{ V}$  vs. SCE at  $25^{\circ}\text{C}$ ); conventional bare electrodes such as glassy carbon, gold, or platinum electrodes require high overpotentials ( $0.6\text{--}0.8 \text{ V}$ ), and its reversible regeneration is a key step in the development of amperometric sensors [2]. These high potentials can cause electrode fouling by oxidation products, loss of sensitivity, repeatability, life time, and the oxidation of other electroactive compounds present in various samples [3]. A decrease of this overpotential can be achieved by modifying electrodes using various electron transfer mediators including phenothiazine redox dyes [4], adenine derivatives [5], Nile blue [6], and thionine [7, 8]. Although the use of mediators can decrease working potential, such biosensors have certain drawbacks including mediator leaching from the electrode surface or lacking of long-term stability in a continuous system [9].

In recent years, with the great progress made in nanoscience and nanotechnology, novel bio-chemical sensor platforms have been developed to enhance the analytical performance of the bio-sensing [10, 11]. There has been significant interest in nanomaterials because of their excellent properties including high electrocatalytic activity, non-toxicity, large specific surface area, and good biocompatibility.

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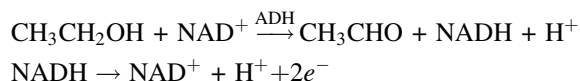
Moreover, numerous nanomaterials such as carbon nanotubes [12], graphene sheets [13], carbon nanofibers [14], Au nanoparticles [15], and metal oxide nanoparticles such as  $\text{Fe}_3\text{O}_4$  [16] and  $\text{TiO}_2$  [17] have been used widely to construct biosensors owing to decreased high overvoltage for electrochemical oxidation of NADH. Therefore, the performance of nanostructure-modified biosensor could be improved efficiently. Among them, with their high electrocatalytic activities, metal oxide nanoparticles can effectively overcome the kinetic barriers for the electrochemical regeneration of  $\text{NAD}^+$ , and thus they improve the electron transfer. NiONPs are considered important metal oxides with attractive electrochemical, thermal, optical, and mechanical properties with their high surface to volume ratio [18, 19].

Recently, NiONPs have received great interest due to unique physical and chemical properties such as electronic, optic, and catalytic [20]. In constructing enzyme-based biosensors, NiONPs are favorable because of their high isoelectric point for physical adsorption of enzyme molecules by electrostatic interactions [21]. The electrochemical applications of NiONPs in glucose [22, 23], myoglobin [24], DNA [25], amino acids [26], and  $\text{H}_2\text{O}_2$  [27] sensors have been also reported and applied in various fields.

Carbon paste electrode (CPE), a mixture of graphite powder and binder (e.g., paraffin/mineral oil), has been used as a matrix since 1958 by Adams for various analytical applications [28–30]. CPEs are becoming popular in electrochemical studies due to their low background current, wide anodic potential range, chemical stability, ease of modification with nanomaterials, and low cost preparation [31, 32]. The integration of metal oxide into carbon paste could provide significant synergic electrocatalytic effects for the detection of NADH.

In this study, we have prepared a carbon paste electrode including NiONPs for the electrochemical oxidation of NADH. The electrochemical properties of NiONPs/MCPE and BCPE were investigated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. Compared to the BCPE, NiONPs/MCPE shows improved electron transfer kinetics due to the nano-size and electrical conductivity of NiONPs. The electrochemical oxidation response of NADH for NiONPs/MCPE was examined by differential pulse voltammetry (DPV) and chronoamperometry (CA) methods. According to the results obtained, this electrode has lower overvoltage and higher electrochemical current responses than BCPE. Several analytical performances including the linear working range, detection limit, and repeatability of the developed NADH sensor were also determined. In addition, NiONPs/MCPE was integrated with a  $\text{NAD}^+$ -dependent alcohol dehydrogenase enzyme to develop of an ethanol sensing system. The alcohol dehydrogenase enzyme (ADH) catalyzes the oxidation of ethanol to acetaldehyde while the cofactors  $\text{NAD}^+$

are reduced to NADH. The enzymatically produced NADH is oxidized at the modified enzyme electrode and can be detected amperometrically according to following equations. The electrocatalytic current is proportional to NADH and ethanol concentration [33].



## 2 Experimental

### 2.1 Materials

Nickel oxide nanoparticles (<100 nm), NADH,  $\text{NAD}^+$ , alcohol dehydrogenase from Baker's yeast in the form of lyophilized powder (ADH, EC 1.1.1.1, 337 U  $\text{mg}^{-1}$ ), glutaraldehyde, bovine serum albumin, and Nafion<sup>®</sup> perfluorinated ion-exchange resin (5 wt% solution) were purchased from Sigma. Graphite powder and paraffin oil were obtained from Fluka. Ethanol was obtained from Merck. Di-sodium monohydrogen phosphate heptahydrate and sodium dihydrogen phosphate dihydrate were acquired from Riedel de Haën. All other chemicals were of analytical grade.

### 2.2 Apparatus and measurements

All electrochemical studies were performed using an AUTOLAB-PGSTAT 302 N electrochemical analyzer (ECO Chemie, Utrecht, The Netherlands) driven by NOVA software (ECO Chemie) in conjunction with a three-electrode cell stand (Bioanalytical Systems, Inc., USA). A conventional three-electrode system was used with BCPE or NiONPs/MCPE as working electrode, a platinum wire (BAS MW 1034) as counter electrode, and Ag/AgCl (3 mol  $\text{L}^{-1}$  KCl, BAS MF 2052) as reference electrode. NiONPs-modified carbon paste alcohol dehydrogenase enzyme electrode (NiONPs/MCPHE) was used as working electrode for the amperometric determination of ethanol.

The electron transfer properties of BCPE and NiONPs/MCPE were investigated in 0.1 mol  $\text{L}^{-1}$  KCl solution containing 5.0 mmol  $\text{L}^{-1}$   $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$  (1:1) by CV and EIS methods. Cyclic voltammograms (CVs) were recorded in potential range between  $-0.2$  and  $0.6$  V at different scan rates. The EIS studies were performed at the frequency range between  $10^5$  and  $10^{-2}$  Hz with 5 mV perturbation at open circuit potential ( $E_{\text{OCP}}$ ).

The effect of NiONPs on the response of NADH was examined by DPV and CA techniques in phosphate buffer solution (PBS, 0.05 mol  $\text{L}^{-1}$ , pH 7.4) supporting electrolyte. Differential pulse voltammograms (DPVs) were

recorded in the potential range between 0.0 and 0.9 V with pulse potential of 50 mV and pulse time of 200 ms.

The determination of NADH was performed using the CA method at +0.4 V potential in 3 mL of phosphate buffer solution (PBS, 0.05 mol L<sup>-1</sup>, pH 7.4) supporting electrolyte under stirring conditions. Once a steady background signal was observed, the NADH solution was successively injected into the PBS and the amperometric current was recorded. For the determination of ethanol, 3 mmol L<sup>-1</sup> NAD<sup>+</sup> was added into the PBS and the same amperometric measurement was performed for NiONPs/MCPEE using ethanol solutions. The pH values of the buffer solutions were measured using ORION Model 1906 D. 720A pH/ion meter (Thermo Scientific USA). The ultrapure water (18.2 MΩ cm) from Purelab Elga system (Veolia Water Systems Ltd., UK) was used for preparing all solutions. All measurements were carried out at room temperature (23 ± 2 °C).

### 2.3 Preparation of bare and NiONPs-modified carbon paste electrodes

The BCPE was prepared by mechanically mixing graphite powder (70 %) and paraffin oil (30 %). NiONPs/MCPEs were composed of NiONPs and graphite powder in various ratios of NiONPs/graphite powder (w/w) and paraffin oil. A portion of the given pastes was placed firmly into a cavity of polyetheretherketone (PEEK) rod electrode bodies (with 3 mm internal diameter), and a brass wire was inserted for electrical contact. The surfaces of the electrodes were smoothened on a weighing paper. The electrodes were stored in the refrigerator at +4 °C when not in use.

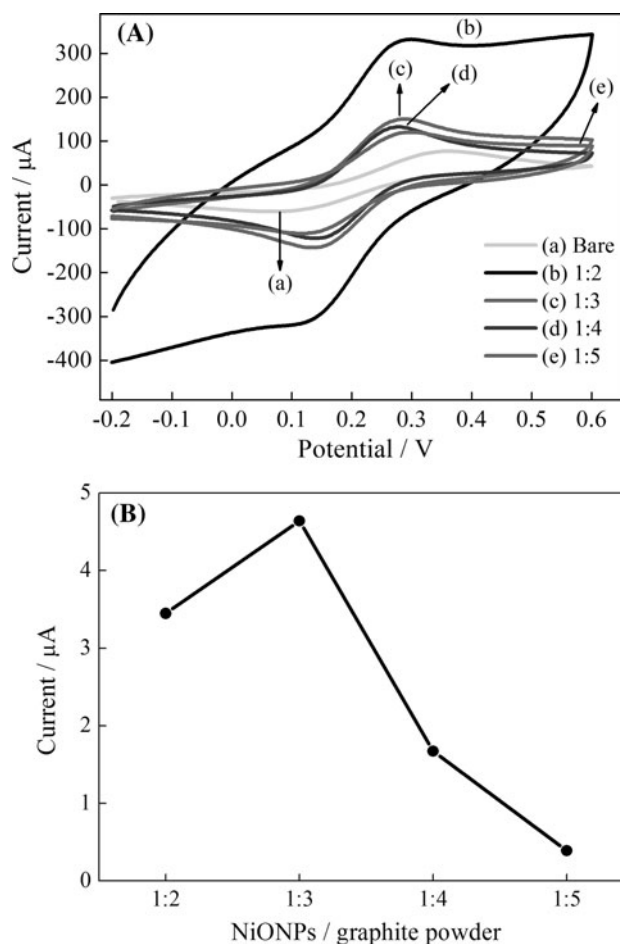
### 2.4 Preparation of ethanol biosensor

Alcohol dehydrogenase enzyme was dissolved in PBS (0.05 mol L<sup>-1</sup>, pH 7.2) and an aliquot of 10 μL; 30 mg mL<sup>-1</sup> enzyme solution was mixed with the NiONPs-graphite powder (1:3) mixture. The paraffin oil was also added to the mixture and the paste obtained was placed into PEEK rod electrode body. The surface of the enzyme electrode was smoothened.

## 3 Results and discussion

### 3.1 The effect of NiONPs amount on the performance of the NiONPs/MCPE

In order to evaluate the effect of the amount of NiONPs on the performance of the electrode, NiONPs/MCPEs were prepared by mixing NiONPs with graphite powder at a



**Fig. 1** **a** CVs of 5 mmol L<sup>-1</sup> [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> on BCPE (a) different NiONPs/MCPEs with the mass ratios of NiONPs to graphite powder were 1:2 (b), 1:3 (c), 1:4 (d) and 1:5 (e) Scan rate: 50 mV s<sup>-1</sup>. **b** The effect of NiONPs amount on the amperometric response of NiO/MCPEs in PBS (0.05 mol L<sup>-1</sup>, pH 7.4) containing 1.0 mmol L<sup>-1</sup> NADH

given ratio (1:5, 1:4, 1:3 and 1:2). The electron transfer properties of the obtained NiONPs/MCPE and BCPE were investigated in [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> redox probe using the CV technique. It has been reported that the electron transfer property of the redox couple is sensitive to the surface chemistry and microstructure as well as the density of electronic states of electrode materials near the Fermi level [34, 35]. The CVs of BCPE and NiONPs/MCPEs in the solution of redox probe showed a pair of redox couples in the potential range of 0.0 and +0.4 V (attributed to the [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> redox process) (Fig. 1a). When NiONPs/MCPEs and BCPE were compared, the peak-to-peak potential separation ( $\Delta E_p$ ) and the current intensities of the [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> for BCPE were larger and lower, respectively. These results indicated that NiONPs provide the improved reversibility of the redox reaction due to their

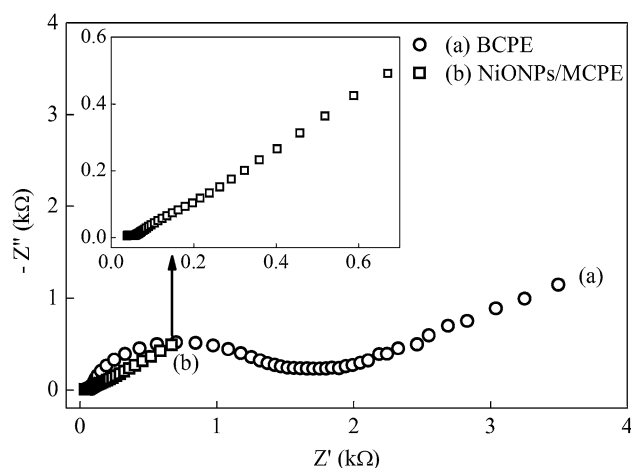
**Table 1** Comparison of electrochemical characteristics of different amount of NiONPs/MCPEs toward the redox probe of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ 

The mass ratio of NiONPs to graphite	$E_{\text{pa}}$ (V)	$E_{\text{pc}}$ (V)	$\Delta E_p$ (V)	$I_{\text{pa}}$ ( $\mu\text{A}$ )	$I_{\text{pc}}$ ( $\mu\text{A}$ )
1:2	0.281	0.134	0.147	101	86
1:3	0.280	0.140	0.140	118	121
1:4	0.278	0.139	0.139	115	114
1:5	0.286	0.130	0.156	82	81

electrocatalytic properties and/or the enlarged electroactive surface area of the modified electrode [36, 37]. With increasing amount of NiONPs to graphite, both cathodic peak current and anodic peak current increased, and  $\Delta E_p$  of the NiONPs/MCPEs were 0.156, 0.139, 0.140, and 0.147 V, respectively. Table 1 also gives the electrochemical characteristics to clarify the electrochemical behaviors of different NiONPs/MCPEs. The NiONPs/MCPEs with the amount of NiONPs to graphite powder of 1:3 and 1:4 demonstrated the relatively smallest  $\Delta E_p$  and largest peak currents, indicating fast electron transfer kinetics. In order to determine the most suitable electrode composition, the amperometric responses of  $1 \text{ mmol L}^{-1}$  NADH were recorded at  $+0.4 \text{ V}$  for each electrode. Figure 1b shows that the best response was obtained when a 1:3 NiONPs/MCPE was used. In NiONPs/MCPE, NiONPs serve as an electrocatalyst for the oxidation of NADH and graphite powder carries the electrons. Therefore, the amounts of graphite powder and NiONPs are important for electrochemical detection of NADH. However, too much incorporated amount of nanoparticles in the paste may lead to undesirable mechanical properties of CPE and increase the resistance of the electrode [35]. Furthermore, the electrons emitted from NADH oxidation cannot be effectively transferred to the electrode in the excessive amount of NiONPs.

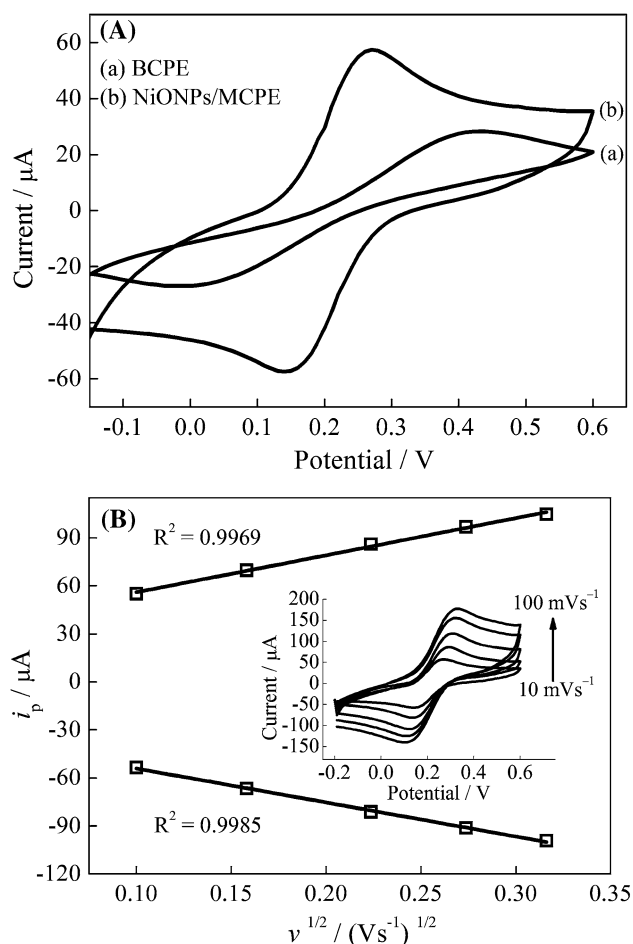
### 3.2 Electrochemical behavior of bare and NiONPs-modified electrodes

Using the NiONPs/MCPE (1:3), the effect of NiONPs on electron transfer features of carbon paste electrode was examined by EIS and CV techniques in  $0.1 \text{ mol L}^{-1}$  KCl containing  $5.0 \text{ mmol L}^{-1}$   $[\text{Fe}(\text{CN})_6]^{3-/4-}$  redox probe. The EIS is an important technique for explaining the interface features of modified electrodes [38]. This technique can also provide useful information about the changes of modified electrode surfaces during the process. Figure 2 shows the electrochemical impedance spectra (Nyquist plots) of BCPE (a), and NiONPs/MCPE (b) in  $5 \text{ mmol L}^{-1}$   $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . The Nyquist plots give a semicircle part at high frequencies

**Fig. 2** The Nyquist plots of (a) BCPE and (b) NiONPs/MCPE in  $0.1 \text{ mol L}^{-1}$  KCl solution containing  $5.0 \text{ mmol L}^{-1}$   $[\text{Fe}(\text{CN})_6]^{3-/4-}$ 

and a linear part at low frequencies. The semicircle part corresponds to the electron transfer limited process and its diameter is equal to electron transfer resistance. The linear part corresponds to the diffusion controlled process [38]. As seen in Fig. 2, the BCPE presents a high electron transfer resistance value ( $\sim 1,500 \Omega$ ). While, the NiONPs/MCPE shows nearly a straight line, indicating that NiONPs demonstrate fast electron transfer kinetics for the  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  redox system [35]. This is attributable to the good electron transfer ability of NiONPs which can facilitate the electrochemical oxidation of NADH on electrode surface.

Figure 3a shows the voltammetric response of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  at BCPE and NiONPs/MCPE. The redox peaks of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  were clearly observed for BCPE and NiONPs/MCPE. When compared to the BCPE, the anodic and cathodic peak current intensities for the NiONPs/MCPE were increased. In addition, while the peak-to-peak separation ( $E_{\text{p,a}} - E_{\text{p,c}} = \Delta E_p$ ) of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  waves for the BCPE were  $440 \text{ mV}$ , the  $\Delta E_p$  for the NiONPs/MCPE was  $130 \text{ mV}$ . These results reveal that the electron transfer at the solution/electrode interface is fast due to the conductivity of NiONPs-modified electrode [39]. The effective surface area of the NiONPs/MCPE was determined from the voltammetric peak current using the Randles–Sevcik equation [38] Eq. (1). The effective surface area of the NiONPs/MCPE was calculated as  $0.155 \text{ cm}^2$  at  $10 \text{ mV s}^{-1}$  scan rates and it is much higher than that of the BCPE ( $0.070 \text{ cm}^2$ ). The current intensity increases due to the enhancement in the surface area of the electrode containing the NiONPs. According to the EIS and CV results, NiONPs facilitate the electron transfer between the redox probe solution and surface of the modified electrode due to high electroactive surface area and the electric conductivity of NiONPs [39].

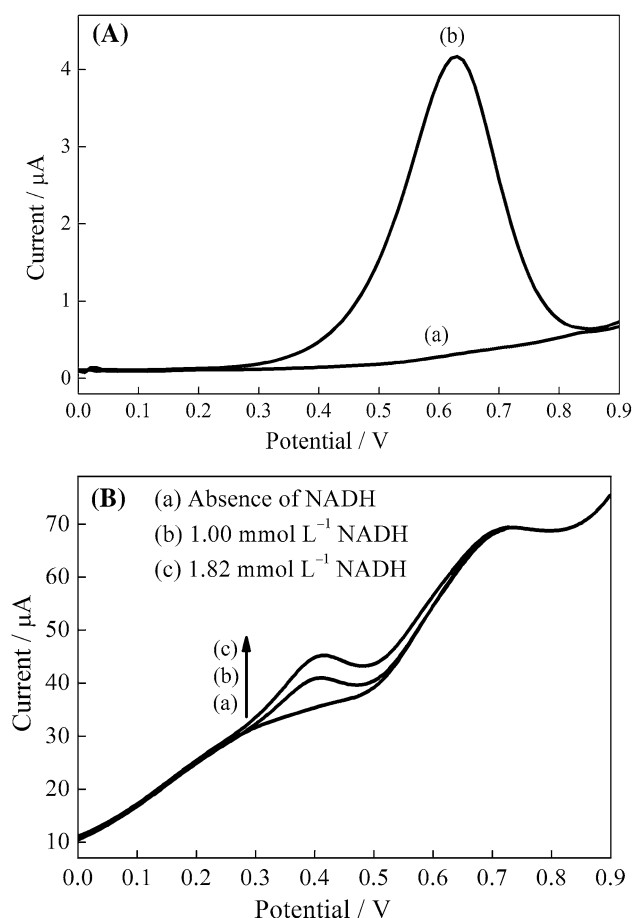


**Fig. 3** **a** CVs of (a) BCPE and (b) NiONPs/MCPE at 10 mV s<sup>-1</sup>. **b** The plot of the peak current versus the square root of the scan rate for NiONPs/MCPE in 0.1 mol L<sup>-1</sup> KCl solution containing 5 mmol L<sup>-1</sup> [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup>. Inset shows cyclic voltammograms at different scan rates

$$i_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} v^{1/2} \quad (1)$$

where  $n$  is the number of electrons involved in the redox reaction,  $A$  is the electrode surface area (cm<sup>2</sup>),  $C$  is the concentration of the redox species in solution (mol cm<sup>-3</sup>),  $D$  is the diffusion coefficient of the molecule in solution ( $6.67 \times 10^{-6}$  cm<sup>2</sup> s<sup>-1</sup> for ferricyanide) [40] and  $v$  is the scan rate (V s<sup>-1</sup>).

The peak current is directly proportional to the concentration in bulk solution considering the equation; it increases with the square root of the scan rate. Figure 3b shows the relationship between the peak current and square root of the scan rate and cyclic voltammograms for [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> redox couple at different scan rates (inset). The plot is linear, suggesting that the mass transfer phenomenon at the solution/electrode interface is mainly a diffusion controlled process [41].



**Fig. 4** **a** DPVs in the absence of and presence of 1.00 mmol L<sup>-1</sup> NADH at BCPE (a, b) and **b** DPVs in the absence (a) of and presence of (b) 1.00 mmol L<sup>-1</sup> and (c) 1.82 mmol L<sup>-1</sup> NADH at NiONPs/MCPE

### 3.3 Electrochemical behavior of NADH

The electrochemical behavior of NADH at the BCPE and the NiONPs/MCPE was examined with DPV in the absence and presence of 1.0 mmol L<sup>-1</sup> NADH. For the BCPE as shown in Fig. 4a, while an oxidation peak was not observed in the absence of NADH, there was an oxidation peak at +0.63 V in the presence of NADH. In the case of the NiONPs/MCPE (Fig. 4b), a broad oxidation peak at +0.69 V was appeared in the absence of NADH, which corresponds to the oxidation of Ni(II)oxide to Ni(III)oxide [42]. In the existence of NADH, two broad oxidation peaks were appeared at +0.41 and +0.69 V. While the second peak (+0.69 V) was assigned to the oxidation to Ni(III)oxide of Ni(II)oxide, the first peak (+0.41 V) was attributed the oxidation of NADH. When the second addition of NADH solution was performed, the oxidation peak at +0.41 V increased. The overpotential for oxidation of NADH at the NiONPs/MCPE decreased 220 mV when compared to that of the BCPE. This



reduction of the overpotential is due to the high electrocatalytic activity of NiONPs, which facilitates the low potential determination of NADH. Therefore, an applied potential of +0.4 V was selected for amperometric determination of NADH.

In the literature, there are many studies about modified electrodes prepared with various materials for NADH detection. Liu et al. [43] used +0.4 V for amperometric sensing of NADH using zirconia nanotubes and poly (acid fuchsin). Different working potentials such as +0.5 V [13], +0.45 V [2, 44], +0.32 V [14], and +0.25 V [45] have been reported for NADH detection. This might be attributed to the electrode modification procedures and electrode material.

### 3.4 Detection of NADH based on NiONPs/MCPE

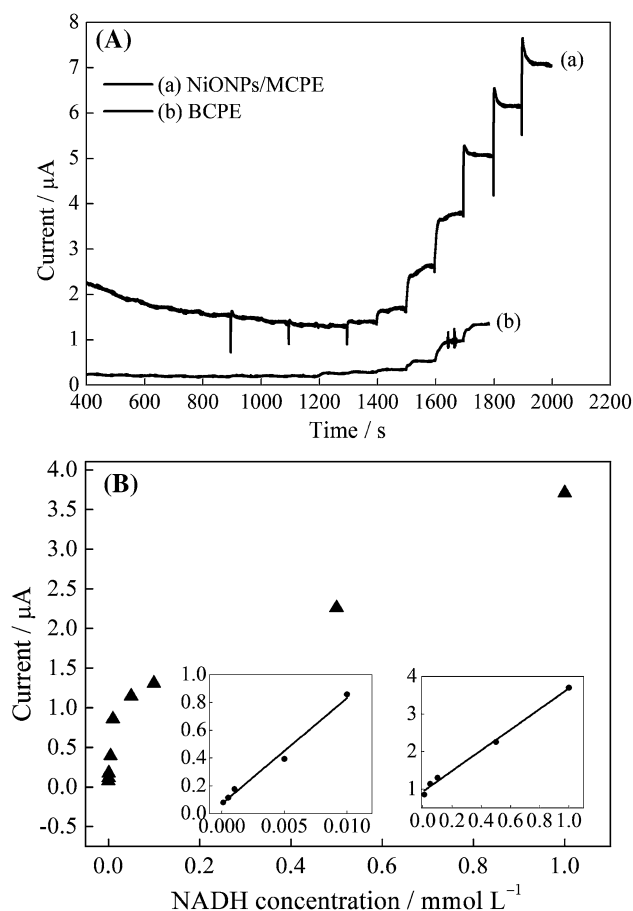
Figure 5a shows the typical amperometric responses of the BCPE and NiONPs/MCPE to successive additions of NADH in 0.05 mol L<sup>-1</sup> PBS (pH 7.4) at the working potential of +0.4 V versus Ag/AgCl. As can be seen in Fig. 5a, the NiONPs/MCPE provides more amplified responses than BCPE. The time required to achieve 95 % of the steady-state current is ~5 s after the addition of NADH. Such a fast response may reflect the increased electron transfer in the NiONPs-modified carbon paste. Figure 5b shows the calibration plot obtained with NiONPs/MCPE. As shown in some recent literature [16, 46], two linear working ranges were obtained. The linear working ranges were  $1.0 \times 10^{-4}$ – $1.0 \times 10^{-2}$  and  $1.0 \times 10^{-2}$ – $1.0$  mmol L<sup>-1</sup> with linear regression equations of  $I (\mu\text{A}) = 76.01 + 0.072 C_{\text{NADH}} (\text{mmol L}^{-1})$  ( $R^2 = 0.9879$ ) and  $I (\mu\text{A}) = 2.75 + 0.94 C_{\text{NADH}} (\text{mmol L}^{-1})$  ( $R^2 = 0.9947$ ), respectively. The limit of detection (LOD) is calculated to be 0.05  $\mu\text{mol L}^{-1}$  ( $S/N = 3$ ). LOD values confirmed the sensitivity of the NiONPs/MCPE which is calculated using following equation:

$$\text{LOD} = 3s/m,$$

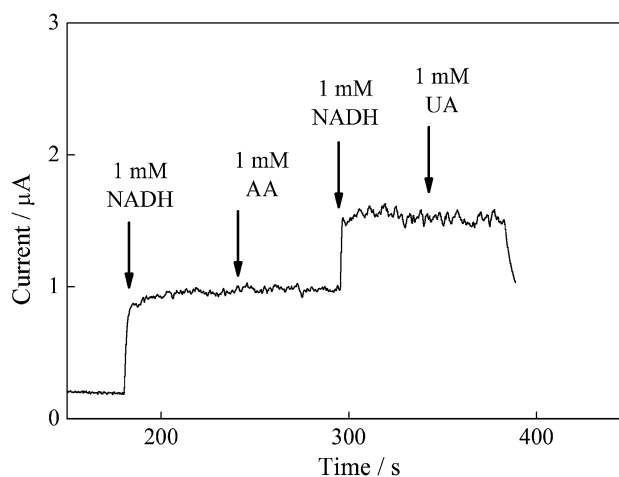
where  $s$  is the standard deviation of the current (five runs) of the lowest concentration of the linearity range,  $m$  is the slope of the related calibration curve.

### 3.5 Interference and repeatability

The effects of common interfering species such as ascorbic acid (AA) and uric acid (UA) on the amperometric response of NiONPs/MCPE to NADH were evaluated (Fig. 6). In order to achieve selective determination of NADH, nafion was dropped onto the surface of NiONPs/MCPE and used as a protective barrier. The addition of 1.0 mmol L<sup>-1</sup> of each of AA (1.0 mmol L<sup>-1</sup>) and UA (1.0 mmol L<sup>-1</sup>) compounds almost did not cause any interference on the NADH response



**Fig. 5** a Chronoamperometric responses of NiONPs/MCPE (a) and BCPE (b) in PBS (0.05 mol L<sup>-1</sup>, pH 7.4) on successive addition of different NADH concentrations at applied potential of +0.4 V (vs. Ag/AgCl). b Calibration plot of chronoamperometric current response versus NADH concentration at NiONPs/MCPE



**Fig. 6** Amperometric response of NiONPs/MCPE in PBS (0.05 mol L<sup>-1</sup>, pH 7.4) containing 1.0 mmol L<sup>-1</sup> NADH spiked with 1.0 mmol L<sup>-1</sup> AA and 1.0 mmol L<sup>-1</sup> UA

**Table 2** Response characteristics of different NADH sensors

Electrodes	Applied potential (V)	Linear range (mmol L <sup>-1</sup> )	Sensitivity (μA/mmol L <sup>-1</sup> cm <sup>-2</sup> )	Detection limit (μmol L <sup>-1</sup> )	Response time (s)
CNF/GCE [14]	+0.32	$3 \times 10^{-2}$ –2.1	3.6	11	3
Graphene/GCE [13]	+0.5	0.05–1.4	12.6 (μA/mmol L <sup>-1</sup> )	20	–
CNF/CPE [2]	+0.45	20–11470	10 (μA/mmol L <sup>-1</sup> )	0.02	5
BGMC/GCE [49]	+0.046	$3 \times 10^{-3}$ –1.4	$1.29 \pm 0.02$ (μA/mmol L <sup>-1</sup> )	1	–
MCMS/PTH/GCE [8]	+0.05	0.002–0.01 0.01–0.1	11.1 (mA/mol L <sup>-1</sup> )	0.51	Less 10 s
IL/graphene/chitosan/GCE [44]	+0.45	0.25–2	37.43	5	20
GNSs/GCE [50]	+0.32	0.002–4.69	535 (mA/mol L <sup>-1</sup> cm <sup>2</sup> )	0.23	–
Polyluminol/MWCNT/GCE [48]	+0.1	$5.0 \times 10^{-3}$ –0.15	183.9	0.6	0.1
FcC <sub>6</sub> SH/MWNTs/GCE[47]	+0.65	$5.0 \times 10^{-3}$ –1.5	–	0.54	–
NiONPs/CPE (This study)	+0.4	$1.0 \times 10^{-4}$ – $1.0 \times 10^{-2}$ $1.0 \times 10^{-2}$ –1.0	76.01 (μA/mmol L <sup>-1</sup> ) 2.75 (μA/mmol L <sup>-1</sup> )	0.05	5

GCE glassy carbon electrode, CNF carbon nanofiber, CPE carbon paste electrode, BGMC bicontinuous gyroidal mesoporous, MWCNTs multi-walled carbon nanotube, MCMS magnetic chitosan microspheres, PTH poly(thionine), IL ionic liquid, FcC<sub>6</sub>SH 6-ferrocenylhexanethiol

indicating that proposed sensor has good selectivity for determining of NADH.

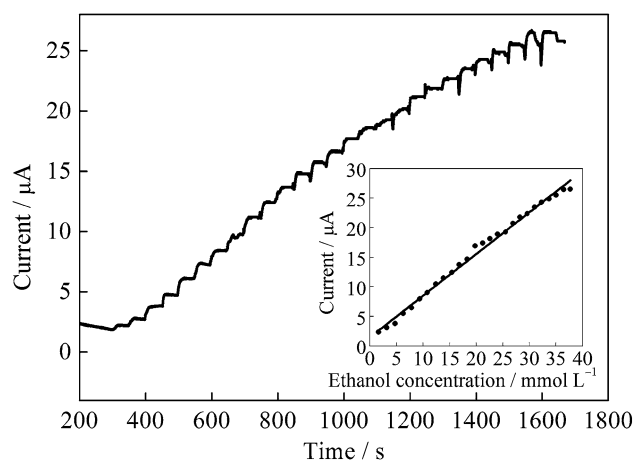
The repeatability of NiONPs/MCPE was examined by amperometric measurements of 1.0 mmol L<sup>-1</sup> NADH at 0.4 V (vs. Ag/AgCl). The relative standard deviation (RSD) was 5.5 % for eight determinations using the same prepared electrode. This result was an indication of good stability.

The characteristics of the proposed NADH sensor in comparison to some of the other NADH sensors reported in the literature are given in Table 2. The results obviously indicate that the proposed NADH sensor based on NiONPs/MCPE shows better analytical performance in terms of detection limit, working range, and sensitivity [13, 44, 47].

The NiONPs/MCPE preparation is easier, less time consuming (~20 min), and repeatable in comparison to the ones described in some of the papers [48, 49]. The electrode developed in this study (modified with a single component) has better properties than electrodes modified with multiple components in the literature. Therefore, this study suggests that NiONPs is a promising candidate for NADH sensing.

### 3.6 Detection of ethanol based on NiONPs/MCPE

Because of the electrocatalytic effect of NiONPs/MCPE toward the amperometric detection of NADH, a dehydrogenase-based amperometric ethanol biosensor is prepared. Figure 7 shows the chronoamperometric response of NiONPs/MCPEE with an applied potential of +0.4 V for additions of ethanol in a stirred 0.05 mol L<sup>-1</sup> PBS (pH 7.4) containing 3 mmol L<sup>-1</sup> NAD<sup>+</sup>. After injecting ethanol in the cell, the current increased and reached a stable state within ~5 s. The current was linear with the ethanol



**Fig. 7** Chronoamperometric response of NiONPs/MCPEE in PBS (0.05 mol L<sup>-1</sup>, pH 7.4) on successive addition of different ethanol concentrations at applied potential of +0.4 V (vs. Ag/AgCl). Inset shows a plot of chronoamperometric current response versus ethanol concentration at NiONPs/MCPEE

concentration over the range from 1.6 to 38 mmol L<sup>-1</sup> with equation of  $I$  (μA) =  $76.01 + 0.072 C_{\text{ethanol}}$  (mmol L<sup>-1</sup>) ( $R^2 = 0.9879$ ) (inset).

## 4 Conclusion

In summary, a new carbon paste electrode was developed and studied. NiONPs-modified carbon paste electrode (NiONPs/MCPE) was made by mixing NiONPs, graphite powder, and paraffin oil. The NiONPs-modified carbon paste electrode preparation technique is easier in comparison to the literature. The characterization of this electrode

was performed by EIS and CV with  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  probe. The new NiONPs/MCPE can be used for amperometric detection of NADH with high sensitivity, good selectivity, and a wide linear working range with a low detection limit at low potential. The NiONPs/MCPE provided a biocompatible microenvironment for ADH and a necessary pathway of electron transfer between ADH and the electrode. Compared to the methods previously reported for electrode modification, this procedure avoids complex synthetic techniques and thereby tenders a simple and cost-effective proposal for the development of  $\text{NAD}^+$  depended dehydrogenase-based biosensors.

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